Risk factors affect prevalence of diarrheal entero-pathogens in children, calves and broiler chickens in Assiut, Egypt

Z.M. Youssef1, S.S. Malek1, S.H. Abo-Elmagd2, R.F. Farghal3 and F.S. Mahmoud1

1Infectious Diseases, Department of Animal Medicine, 2Zoonoses, Department of Zoonoses, 3Poultry Diseases, Department of Avian and Rabbit Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt

Abstract

Diarrhea is a complex syndrome causing colossal economic losses. The objective of the current study was to diagnose Enter-pathogens in children, calves, and broiler chickens by a serological and molecular diagnosis of Rotavirus, evaluate Escherichia coli (E. coli) and Salmonella spp. with bacteriological examination beside conventional diagnosis of Cryptosporidium in addition to studying risk factors affecting these pathogens in Assiut Governorate. From February 2021 to April 2021, a total of 60 samples were collected from the stool of children (n=20), feces of calves (n=20) in addition to liver and pool intestine of broiler chickens (n=20) were examined by Immuno Chromatographic Assay (ICA), Reverse Transcriptase Polymerase Chain Reaction (RT-PCR), microbiological examination and modified Ziehl-Neelsen stain smears.

The prevalence of Rotavirus infection by ICA was 55% (11/20), 10% (2/20), and 5% (1/20) of examined children, calves, and broiler chickens, respectively. Two (18.18%) of serologically Rotavirus positive stools of children were positive molecularly by RT-PCR, while serologically positive fecal and pool intestines of calves and broiler chickens, respectively, were negative molecularly. There was no significant variation between Rotavirus, E. coli, and Cryptosporidium infections with gender and age of investigated children and between these enteropathogens with sex, age, and species of examined calves. In addition, there were no significant differences between Rotavirus, E. coli, Salmonella, and Cryptosporidium with the age of investigated broiler chickens. It is concluded that these enteropathogens in livestock and chicken living close to the human population necessitate better surveillance and control measures to protect vulnerable animal and human populations.

Keywords: Rotavirus, E. coli, Cryptosporidium

Introduction

Livestock plays an outstanding role in the national economy and the socio-economic development of millions of rural households (1). Enteritis is a significant problem in livestock production in Egypt and worldwide (2). Fecal animals are a source of enteritis infection in young children in low- and middle-income countries by contamination of their food (3,4). Diarrhea is a complex multi-factorial etiology, usually influenced by environmental, management, nutritional, and physiological variations and different pathogens, including Rotavirus, E. coli, Salmonella, and Cryptosporidium that are described as essential agents causing enteritis (either separately or in combination) (2). The Rotavirus belongs to the genus Rotavirus, family Reoviridae, has 11 segments of double-stranded ribonucleic acid (dsRNA) in its genome, contains triple layered capsid protein, and encodes six viral structural proteins (VP) (VP1-VP4, VP6, and VP7) and six non-structural proteins (NSP) (NSP1-NSP6) (5). Depending on VP6, Rotaviruses are
classified into ten serogroups (A-J) (5). Rotavirus is the major etiological agent causing acute gastroenteritis in different species of mammals (6). Avian Rotavirus is morphologically identical and antigenically related to mammalian Rotavirus, and it is like mammalian Rotavirus in its genome (6). An avian-like group A Rotavirus has been isolated from enteric calf feces, and avian Rotavirus has been found to induce diarrhea in mammals (6). Laboratory diagnosis of Rotavirus enteritis is based on detecting viral antigens and nucleic acids in stool, fecal, and pool of intestine samples. Immune chromatographic assay ICA is a rapid and sensitive test requiring a shorter time than viral isolation for Rotavirus antigen detection (5,7). Virus confirmation can be performed at the genomic level using the molecular method of RT-PCR (7). E. coli and Salmonella belong to the family Enterobacteriaceae and are the most common and economically critical bacterial agents (2,8). Zoonotic enteropathogens include a wide range of microorganisms transmitted to humans by consuming dairy or meat products, food or water contaminated with animal feces, or direct contact with animal feces in the environment (3). Animals carry pathogenic E. coli strains that can cause gastrointestinal illness in humans, and they play an essential role in fecal contamination of sources of drinking water and crops that enable the direct transfer of zoonotic organisms to humans (9). E. coli infection is an important bacterial avian pathogen responsible for various disease syndromes in farmed birds, causing 5-50% mortality in the poultry industry (10). Several strategies have been adapted to isolates of E. coli by culturing that aiding the identification of pathogenic strains in humans, animals, and poultry (3,9,10). Salmonellosis is a food-born infection with worldwide importance and is a zoonotic bacterial disease that causes economic problems in both developed and developing countries (11). Salmonella infection can be diagnosed by identification of this bacteria in animals, humans, and poultry (11). The gold standard for Salmonella detection is bacteriological culture (11). Cryptosporidium is a common enteric protozoa that belong to the phylum Apicomplexa (12). It is a coccidian protozoan that infects various vertebrate animals, humans, and birds (12). Cryptosporidium is most common in pre-weened calves, resulting in clinical disease with the potential of zoonotic transmission to human contacts like children (12,13). On the other hand, birds such as chickens are infected with several Cryptosporidium species. Chicken acts as a reservoir for transmission to humans (12). Cryptosporidium infection in farm animals and humans is diagnosed under microscopy by detecting oocysts in smears by conventional techniques such as the modified Ziehl Neelsen staining method (12,13).

The present study aimed to investigate the presence of enteropathogens (Rotavirus, E. coli, Salmonella and Cryptosporidium) in children, calves, and broiler chickens by a serological and molecular diagnosis of Rotavirus. Also, evaluation of E. coli and Salmonella with the bacteriological examination. Besides, perform the conventional diagnosis of Cryptosporidium in them. Correlation between different factors, such as gender/sex, age, and species of children, calves, and broiler chickens with infection rates of these enteropathogens, was also assessed in Assiut Governorate/Egypt.

Materials and methods

Ethical approval

All study conditions were approved by the Research Ethical Committee of the Faculty of Veterinary Medicine, Assiut University, Assiut, Egypt (aun/vet/3/0007).

Human, calves, and broiler chickens sample collection

A total of 60 samples were collected from the stool of children, feces of calves, and liver and pool intestine of broiler chickens during the period of investigation between February 2021 and April 2021. For the humans, stool samples were obtained from 20 children (11 male and 9 female) of variant ages that were in contact with 20 calves (7 male and 13 female) of different ages and species. For broiler chickens, liver and pools of intestine were collected from 20 broiler chickens aged from 20 - 36 days from areas that overlapped with calves and human beings in the North, Center, and South of Assiut governorate/Egypt. Clinical findings of investigated children were gastrointestinal tract disturbances like vomiting and diarrhea with fever, and examined calves suffered from diarrhea. In addition to investigated broiler, chickens showed diarrhea, depression, pasty vent, and ruffled feathers with swollen eyes.

Serological detection of Rotavirus antigen by ICA (Rotavirus rapid test device)

Sixty samples (20 feces of calves, 20 stools of children, and 20 pools of intestine of broiler chickens) were subjected to ICA for detection of Rotavirus antigen by a commercial kit (Atlas Medical, United Kingdom).

RNA extraction

The positive Rotavirus samples were prepared by 50% stool of children, fecal of calves, and pool of intestine of broiler chickens' suspension (w/v) in phosphate buffer saline was clarified by centrifugation at 12000 rpm for 30 min and supernatant was used for RNA extraction (14). Viral RNA extraction was performed using a QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

RT-PCR

The extracted RNA was denatured at 97°C for 3 minutes and immediately placed on ice (7). Then, OneStep RT-PCR was performed using Qiagen OneStep RT-PCR Kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions. The primers pair was used to amplify the Viral
structural protein 7 (VP7) gene of *Rotavirus*. The primers sequences were as follows: an upstream primer: 5’- GGC TTT AAA AGA GAG AAT TTC CGT CTG G-3’, downstream primers: 5’- GGT CAC ATC ATA CAA TTC TAA TCT AAG -3’ (7.15). The amplified products of *Rotavirus* of 1062 bp were visualized on 1.5% agarose gel stained with Ethidium bromide and photographed by a gel documentation system (BDA digital Biometra, Germany).

**Isolation of *E. coli***

*E. coli* isolation was done according to Lee and Nolan (16). Collected stool of children, feces of calves, and liver samples of broiler chickens were cultured on tryptic soy broth (Oxoid CM129) and incubated at 37°C for 18-24 hours. A loop full of incubated broth was streaked Eosin Methylene Blue (EMB) agar (Oxoid CM69) that was used to observe bacterial growth and incubated at 37°C for 18-24 hours.

**Isolation of *Salmonella***

*Salmonella* isolation was done according to ISO (17). Rappaport-Vassiliadis Enrichment Broth (RVS) (Himedia) was used to isolate *Salmonella* from the stool of children, feces of calves, and pool intestine of broiler chickens incubated at 37 °C for 18 hours. A loop full of culture was streaked onto Xylose Lysine Deoxycholate agar (XLD) (Oxoid CM0469) and incubated at 37 °C for 18-24 hours.

**Identification of *E. coli* and *Salmonella***

Suspected colonies for *E. coli* and *Salmonella* were subjected to morphological and biochemical identification according to MacFaddin (18).

**Lactose fermentation**

Lactose fermentation was examined by culturing a single suspected pure colony on MacConkey agar (Himedia M081) and incubated at 37 °C for 24 hours; a rosy/pink colony was indicative of lactose fermentative bacteria, while the pale colony was indicative of non-lactose fermentative bacteria.

**Triple Sugar Iron (TSI) agar**

The heavy inoculum was stabbed into the bottom and streaked over the surface of the slope of TSI agar (Oxoid CM0277), incubated aerobically at 37 °C for 24 hours. Interpretation of the results was recovered by changing colors at the surface and the bottom, with or without H₂S and gas production.

**Identification of *Cryptosporidium* oocysts**

Direct smears were prepared from stool, fecal, and pool intestine specimens of children, calves, and broiler chickens, respectively, and processed for microscopical examination after staining of smears with modified Ziehl-Neelsen stain according to Bessat *et al.* (12), and oocysts were identified by examining the stained smears under the 100X-oil immersion objectives of the optical light microscope (Optika, Italy).

**Statistical analysis**

The obtained results were analyzed by Chi-square of independence to the association between prevalence and different parameters (gender/sex, age, and species) by using the Statistical package for the social sciences (SPSS) version 16 software program.

**Results**

**Prevalence of enteropathogen infection among children, calves, and broiler chickens**

In the present study, a total of 60 examined samples for *Rotavirus* antigen, 14 (23.33%) were serologically positive by ICA as indicated by a different red band (Result line) appearing in the white central zone of the test (Result region) beside the green band (Control line) (Figure 1). The prevalence of *Rotavirus* infection by ICA was 55% (11/20), 10% (2/20), and 5% (1/20) of investigated children, calves, and broiler chickens, respectively (Table 1). Two (18.18%) of the serologically positive stool of children were molecularly positive for *Rotavirus* by RT-PCR (Figure 2), while serologically positive fecal and pool intestines of calves and broiler chickens, respectively, were molecularly negative (Figure 2). The specific band showed 1062 bp after PCR amplification of the VP7 gene of *Rotavirus* group A. Culturing of suspected *E. coli* samples on EMB agar showed flat dark colonies with a green metallic sheen. The suspected colonies on MacConkey's agar showed medium-sized, rounded, and pink colonies that act as identification for lactose fermentation (Figure 3). In addition, the biochemical test of suspected colonies showed a yellow butt and slant with gases on TSI (Figure 4). The prevalence of *E. coli* infection was 10% (2/20), 10% (2/20), and 55% (11/20) of examined children, calves, and broiler chickens, respectively (Table 1). Colonies of suspected Salmonella culture appeared pink with a black center on XLD agar. On MacConkey's agar showed pale colonies (non-lactose fermenter) (Figure 3). Biochemical identification of cultures showed a yellow butt and red slant with H₂S (blackening) on TSI (Figure 4). Prevalence of *Salmonella* infection was detected only in broilers by 35% (7/20), while it did not find in children and calves (Table 1). Microscopic examination of stool, fecal, and pool intestine smears stained with modified Ziehl-Neelsen stain revealed the characteristic diagnostic stages of *Cryptosporidium* as acid-fast-stained oocysts on a blue background (Figure 5). The prevalence of *Cryptosporidium* in investigated children, calves, and broiler chickens was 10% (2/20), 40% (8/20), and 20% (4/20), respectively (Table 1). According to single infection in (Table 2), *Rotavirus* infection was most common in children, while *Cryptosporidium* infection was in calves. Single infection with *E. coli* and *Salmonella* was prevalent in broiler...
chickens. Regarding double mixed infection, Rotavirus with E. coli was observed in calves, while Rotavirus with Cryptosporidium and E. coli with Cryptosporidium were recorded in children. In addition, E. coli with Salmonella and Salmonella with Cryptosporidium were reported in broiler chickens (Table 2). According to triple mixed infection, Rotavirus, E. coli, and Cryptosporidium were found in calves, while E. coli, Salmonella, and Cryptosporidium were shown in broiler chickens (Table 2).

Table 1: Number and percentage of enter pathogens in investigated children, calves, and broiler chickens

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total number</th>
<th>Rotavirus</th>
<th></th>
<th>E. coli</th>
<th></th>
<th>Salmonella</th>
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<th>Cryptosporidium</th>
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</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
<td>+ve</td>
<td>%</td>
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<tr>
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<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Calves</td>
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<td>10</td>
<td>2</td>
<td>10</td>
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<td>0</td>
<td>8</td>
<td>40</td>
</tr>
<tr>
<td>Broiler Chickens</td>
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<td>5</td>
<td>11</td>
<td>55</td>
<td>7</td>
<td>35</td>
<td>4</td>
<td>20</td>
</tr>
</tbody>
</table>

Figure 1: Rapid Test device for detection of Rotavirus antigen: Positive result (In addition to the green band (Control line), a different red band (Result line) appears in the white central zone of the test (Result region). (A): Children’s stool sample, (B): Calf fecal sample, and (C): broiler chicken pool intestine sample.

Figure 2: Agarose gel electrophoresis of RT-PCR after amplification of VP7 gene of Rotavirus. Children samples, Lane M: DNA Marker of 100 bp, Lane 1: Control positive sample, Lanes 2 and 3: Positive samples of Children with an amplified product at 1062 bp.
Table 2: Number and percentage of enteropthogens in investigated children, calves, and broiler chickens

<table>
<thead>
<tr>
<th>Variable</th>
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<th>Children %</th>
<th>Calves No.</th>
<th>Calves %</th>
<th>Broiler Chickens No.</th>
<th>Broiler Chickens %</th>
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<td>5</td>
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<tr>
<td></td>
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<td>5</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>Salmonella only</td>
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<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>Cryptosporidium only</td>
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<td>0</td>
<td>7</td>
<td>35</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Rotavirus + E. coli</td>
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<td>0</td>
<td>1</td>
<td>5</td>
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<td>0</td>
</tr>
<tr>
<td>Double infection</td>
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<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli + Cryptosporidium</td>
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<td>5</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>E. coli + Salmonella</td>
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<td>0</td>
<td>0</td>
<td>3</td>
<td>15</td>
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<tr>
<td></td>
<td>Salmonella + Cryptosporidium</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Triple infection</td>
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<td>0</td>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>E. coli + Salmonella + Cryptosporidium</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

Figure 3: Colonies of isolated bacteria on MacConkey’s agar. (A): Non-lactose fermenter (yellow colonies): *Salmonella* and (B): Lactose fermenter (pink colonies): *E. coli*.

**Prevalence of different enteropathogens in relation to sex and age of investigated children**

Concerning sex susceptibility, the results revealed no significant difference between male and female children to infection by *Rotavirus*, *E. coli*, and *Cryptosporidium*. However, mathematically male children 63.64% had a higher infection rate with *Rotavirus* than females 44.44%, but female children 11.11% had a higher infection rate with *E. coli* than males 9.09% (Table 3). According to the aging susceptibility of children to infection with *Rotavirus*, *E. coli*, and *Cryptosporidium*, there was no significant variation between different age groups and these enteropathogens. However, mathematically age group 6 months - 1 year of children 60% was more infected with *Rotavirus* than other age groups, while the age group> 1 year was more infected with *E. coli* 25% and *Cryptosporidium* 50% (Table 3).

Figure 4: Inoculated isolated colonies on TSI agar. (A): *E. coli* and (B): *Salmonella*.

Figure 5: Stool, fecal, and pool intestine smears stained with modified Ziehl-Neelsen stain showing oocysts of *Cryptosporidium* spp. with 100X. (A): Children’s stool sample, (B): Calf fecal sample, and (C): Broiler chicken pool intestine sample.
Diarrhea is a common syndrome caused by management, environmental, nutritional, and physiological differences and variants of enteropathogens such as Rotavirus, E. coli, Salmonella, and Cryptosporidium are described as critical infectious agents causing enteritis (either single or mixed) (18). Defining the map of children, calves, and broiler enteritis-causing enteropathogens allows for the application of effective preventative measures. In our study, the prevalence of Rotavirus infection by ICA was 55% (11/20) of examined children, and this finding was higher than recorded by Biswas et al. Simo-Fouda et al. (19,20), who elucidated that the prevalence of Rotavirus infection in investigated children was 51% and 7.7%, respectively. On the contrary, the highest rate of Rotavirus infection in investigated children was offered by Shafik et al. (21), that mentioned that Rotavirus infection was detected in 82% of examined children. In the current study prevalence of Rotavirus infection by ICA was 10% (2/20) of investigated calves, and this finding was higher than that obtained by Abdel-Rady et al. (22), who found that Rotavirus infection was observed in 8.54% of examined calves.

In contrast, the highest rate of Rotavirus infection in investigated calves was concluded by Abdulazezz and Abed, Youssef and Zaitoun (23,24), which revealed that the prevalence of Rotavirus infection in examined calves was 53.6 and14.92%, respectively. In the present study, the prevalence of Rotavirus infection by ICA was 5% (1/20) of investigated broiler chickens. This result was higher than that reported by Wani et al. (6), who mentioned that the prevalence of Rotavirus infection was 4% (3/75) of examined broiler chickens, but this finding was lower than the results observed by Karim et al. (25), Kattoor et al. (26), Pauly et al. (27) that found that the prevalence of Rotavirus infection was 13.81%, 15% and 36.1% in investigated chicken. In the present work, the prevalence of E. coli infection was 10% (2/20) in examined children, and this finding was lower than recorded by Vasco et al. (3), which reported that the prevalence of E. coli infection was 17.20% in investigated children. In the present result, the prevalence of E. coli infection was 10% (2/20) in examined calves,
which was lower than that obtained by Merera et al. (1), and Vasco et al. (3), whom recorded that the prevalence of *E. coli* infection was 28.60% and 50.30% in examined calves. In the current result, the prevalence of *E. coli* infection was 55% (11/20) of investigated broiler chickens, and this result was higher than shown by Vasco et al. (3) Zahid et al. (10) that obtained that prevalence of *E. coli* infection was 7.10% and 45% in examined chickens but lower than reported by Ezz el-Deen et al. (28) who isolated *E. coli* from chickens with the prevalence of 75%. In our data, the prevalence of Salmonella infection was detected only in broiler chickens by 35% (7/20), and this result was higher than that obtained by Khan et al. (29), who isolated Salmonella in 20.50% of examined chickens but lower than reported by Byomi et al. (11) that mentioned that prevalence of Salmonella in investigated chickens was 71.15%. In the current study, the prevalence of Cryptosporidium oocysts in investigated children and calves was 10% (2/20) and 40% (8/20), respectively. These findings were higher than that previously obtained by Abu Samra et al. (30), that concluded that the prevalence of Cryptosporidium oocysts in examined children and calves was 5.60% and 0.6%, respectively, but lower than previously recorded by Bessat et al. (12) that showed the prevalence of Cryptosporidium in children and calves was 16.60% and 43.20%, respectively. In our data, the prevalence of Cryptosporidium in examined broiler chickens was 20% (4/20), and this result was higher than reported by Bessat et al. (12), and Cao et al. (31), they noted that the prevalence of Cryptosporidium in examined chickens was 6% and 2.43%, respectively but lower than concluded by Jarad (32) who recorded that prevalence of Cryptosporidium in investigated chickens was 51.40%. Such variations in the rate of infection of Rotavirus, *E. coli*, Salmonella, and Cryptosporidium in examined children, calves, and broiler chickens may be attributed to the clinical phase of the disease is a suitable time for sampling collection to obtain the better result from a test, hygienic measures and environmental conditions.

In our study, two serologically Rotavirus-positive stools of children were molecularly positive by RT-PCR after amplification of the VP7 gene of Rotavirus group A while the serologically positive fecal samples of calves and pool intestine of broiler chickens were molecularly negative. This finding may be ascribed to three plausible reasons; a) non-specific inhibition of PCR reaction by the components of fecal and intestinal content samples, b) mismatches in primer binding sites or c) the sequence variation in Rotavirus genome due to the segmented nature of RNA genome, Rotavirus is continuously changing and leading to the emergence of new genotypes (7) or Rotaviruses that possess an antigenic A group is considered typical of mammals, while typical avian Rotaviruses are those designated as groups D, F, and G (33).

According to Single infection, Rotavirus infection was the most common in children, and this result was in agreement with Shrivastava et al. (34), and Msolo et al. (35), whom observed that Rotavirus infection was more prevalent enteropathogens in children. This finding may be attributed to Rotavirus remains one of the peak causes of childhood diarrhea which causes hospitalizations and mortalities in children every year, especially in impoverished countries, despite improvements in the development of vaccines for Rotavirus over the years (35). Regarding double mixed infection, Rotavirus with Cryptosporidium and *E. coli* with Cryptosporidium were recorded in children, and these findings were similar to those previously reported Shrivastava et al. (34), and Msolo et al. (35). These results may be explained by coinfection that causes an exacerbated diarrheal condition, and there was a synergistic effect between Rotavirus and Cryptosporidium beside *E. coli* and Cryptosporidium on diarrheal disease in addition to supports the above fact where virus- parasite and bacterial parasite co-infections are the aggravating factors of severe diarrhea in children (34,35). Concerning Single infection, Cryptosporidium infection was in calves, and this result was matched with Abou EL-Ella et al. (36), and Wei et al. (37). This finding may be attributed to several factors, such as the tremendous proliferative capacity of protozoa and resistance of oocysts, besides poor hygiene and sanitary conditions found on much ruminant exploitation, which lead to the presence of the parasite in regions that raise sheep and goats, which serve as asymptomatic carriers of the disease (36). In referring to double mixed infection, Rotavirus with *E. coli* was observed in calves, and this finding was in agreement with Uhde et al. (38), Içen et al. (39) and Smith (40). According to triple mixed infection, Rotavirus, *E. coli*, and Cryptosporidium were found in calves, which were similar Abou EL-Ella et al. (36), Wei et al. (37), Uhde et al. (38), and Içen et al. (39). These results may be due to Rotavirus damage small intestine that facilitates systemic invasion by normal intestinal flora, including *E. coli*, and colonization of the small intestine by *E. coli* has been associated with impaired glucose and lactose absorption, decreased serum glucose concentration and possibly increased susceptibility to Cryptosporidium infection (41). Regarding single infection, single infection with *E. coli* and Salmonella were prevalent in broiler chickens, and these findings were parallel with Shaltout et al. (42). According to double mixed infection, *E. coli* with Salmonella and Salmonella with Cryptosporidium were reported in broiler chickens. Concerning triple mixed infection, *E. coli*, Salmonella and Cryptosporidium were shown in broiler chickens, and these results were matched with Hauck (43). These findings may suggest a synergism between Cryptosporidium and the bacteria such as *E. coli* and Salmonella (43).

Focusing on the effect of gender, the results revealed no significant difference between male and female children to infection by Rotavirus, *E. coli*, and Cryptosporidium. However, mathematically male children had a higher infection rate with Rotavirus than females, and these findings
were similar to that of Biswas et al. (19), Shafik et al. (21), Ibrahim et al. (44), and Kalantari et al. (45). This finding may be attributed to the high possibility of males being infected with Rotavirus than females to the tendency of parents to take care of males than females concerning the treatment in hospitals, and the hypothesis that females are more resistant to infection than males due to hereditary factors represented in XX chromosomes in females (21). In our result, female children had a higher infection rate with E. coli than males, and this was in agreement with Gould et al. (46). This result matched a previous study by Chang and Tserenpuntsag (47), who recorded that females were more likely than male children to develop enteritis by E. coli. According to the aging susceptibility of children to infection with Rotavirus, E. coli, and Cryptosporidium, there was no significant variation between different age groups and these enteropathogens. However, mathematically, the age group 6 months - 1 year of children 60% was more infected with Rotavirus than other age groups, and this finding agreed with Ibrahim et al. (44), Ojobor (48), and Shrestha et al. (49). This result may be due to the high exposure of children in this age group to contaminated materials, and children in this group were known to consistently put their hands into their mouth beside passive immunity acquired by infants from their mothers protected from Rotavirus gastroenteritis during 0-6 months of age, these immunities disappear post 6 months of infant’s age (21,44). In our study, the age group> 1 year of children was more infected with E. coli 25% and Cryptosporidium 50%, and these findings were parallel with Al-Alousi and Mahmood (13), and Ochoa et al. (50). These results may be attributed to the children at the weaning period being exposed to a contaminated environment, food, and water and, after six months of age, increased exposure to enteropathogens as potentially contaminated foods are introduced into the diet (13,50).

According to the sex susceptibility of investigated calves to different enteropathogens, the present study reported no significant difference between sex and Rotavirus, E. coli, and Cryptosporidium. However, mathematically male calves were more infected with Rotavirus 14.29%, E. coli 14.29%, and Cryptosporidium 57.14% than female calves. These results were similar to Merera et al. (1), Youssef and Zaitoun (24), and Mahmoud et al. (51). These findings may be attributed to male calves not being as needed in the production system, and producers mainly weaned the female calves for replacements (1). Regarding the aging susceptibility of examined calves to Rotavirus, E. coli, and Cryptosporidium, statistically, there was no significant variation between different age groups and these enteropathogens. However, mathematically age group > 45 - 3 months had a higher infection rate with Rotavirus 16.67%, E. coli 16.67%, and Cryptosporidium 50% than the age group 4 - 45 days. These findings agreed with Aminu et al. (52) finding that older calves had a higher prevalence rate with Rotavirus than younger ones, and also Awad et al. (53) recorded the presence of E. coli in enteric older calves till 12 weeks. In addition, Bessat et al. (12), and Wegayehu et al. (54), recorded those older calves had a higher infection rate with Cryptosporidium than younger calves. These results may be attributed to the fact that young calves can be protected from Rotavirus more than older calves through maternally derived antibodies. The possible explanation for E. coli in older diarrheic calves may be attributed to concurrent infection with other enteropathogens (52,53).

Concerning to species susceptibility of investigated calves, there was no significant difference between cattle and buffalo calves to different enteropathogens, although mathematically, cattle calves had higher infection with Rotavirus 14.28%, E. coli 14.28% and Cryptosporidium 42.86% than buffalo calves. These findings were previously recorded by Youssef and Zaitoun (24), Mahmoud et al. (51), and Awad et al. (53), in which the susceptibility of cattle calves to be infected with enteropathogens was higher than buffalo calves. This may relate to the difference in natural immunity of the two species, and buffalo calves had strong body immunity (24.54).

Regarding the aging susceptibility of broiler chickens, there was no significant variation between the age groups of investigated broiler chickens and different enteropathogens. However, mathematically age group 20 - 30 days was more susceptible to infection with Rotavirus 11.11%, E. coli 55.56%, Salmonella 33.33%, and Cryptosporidium 22.22% than the age group > 30 - 40 days. These findings were previously reported by Abd El-Mohsen et al. (8), Karim et al. (25), Matin et al. (55), and Guechtiouli et al. (56), they concluded that younger chickens could be more infected with Rotavirus, E. coli, Salmonella, and Cryptosporidium than older chickens. The probable reasons for Rotavirus may be due to a meager amount of virus in samples or the absence of Rotavirus particles in samples of age group > 30 - 40 days (25). Our finding of E. coli may be attributed to colibacillosis, usually seen in young chicks up to three weeks of age, besides immunosuppression and viral infection acting as predisposing factors for this disease (57). Our result of Salmonella may be due to intestinal colonization of Salmonella, invasion of internal organs and persistence in colonized tissues are all higher in newly hatched chicks than in older birds due to their acquisition of protective microflora that either compete with Salmonella for intestinal receptors or produces negative factors (8). The explanation of our finding of Cryptosporidium may be attributed to the poor hygienic conditions at the hatchery as a large percentage of chicks were affected by Cryptosporidium in age groups 20 - 30 days. Furthermore, these may have favored the infestation through their debilitating action on the immune system in addition to lower infestation with Cryptosporidium at age group > 30 - 40 days due to the resistance of broiler chicken, which likely has antibody levels against Cryptosporidium sufficient to counteract the infection at this age (56).
Conclusion

According to the results of this study, Rotavirus, E. coli, Salmonella, and Cryptosporidium play a role in the etiology of enteritis in children, calves, and broiler chickens. Identification of the possible causative agent of diarrhea is crucial because it allows knowing possible risk factors or sources of infection. It is worth applying to field diagnosis for Rotavirus, E. coli, Salmonella, and Cryptosporidium.

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Conflict of interest

The authors declare that there are no conflicts of interest.

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Zubin Mohamed Yousef, صفاء ماجد مالك، شهيرة حامد محمد، زينب محمد يوسف، فاطمة صابر محمود، مالك يحيى جبر، راجي فاروق فرغلي، و فاطمة صابر محمود

الأمراض المعدية، قسم طب الحيوان، أمراض مشتركة، قسم الأمراض المشتركة، أمراض الدجاج، قسم طب الطيور والأرانب، كلية الطب البيطري، جامعة أسيوط، أسيوط مصر

الخلاصة

نستعرض دراسات عديدة تم إجراؤها على حيوانات الأليف والأرانب، وذلك من أجل تحديد مدى انتشار مرض عوامل الخطر المؤثرة على مرض الإسهال المعوية لدى الأطفال والجُدُح ودجاج التسمين في مصر. نحن نعمل على تحديد مركبات المخاطر المحتملة للإصابة في حالة الإسهال المعوية ونتناول هذه النتائج في الختام.


المجهرية ومسحات صبغة زيل-نيلسن المحورة وجد أن معدل انتشار الإصابة بفيروس الروتا و باستخدام المقايسة المناعية 55% (20/20)، 10% (2/20) و 5% (1/20) من الأطفال المختبرة، العجول ودجاج التسمين، على التوالي، ظهرت عينتان فقط 18.18% من عينات البراز الأطفال إيجابية لفيروس الروتا بوساطة تفاعل البلمرة المتسلسل العكسي بينما كانت عينات البراز والأمعاء الخبيئة لفيروس الروتا الخاصة بالعجل والدجاج على التوالي سلبية بوساطة تفاعل البلمرة المتسلسل العكسي.

لم يظهر اختلاف معنوي بين عدوى فيروس الروتا العصبات القولونية والأبواغ الخبيئة مع الجنس والعمر للأطفال وأيضًا بين البكتريا المعوية مع الجنس والعمر وقصب الصور المختبرة بالإضافة إلى عدم وجود فرق معنوي بين فيروس الروتا، العصبات القولونية والسالمونيلا والأبواغ الخبيئة مع عمر دجاج التسمين المختبرة. ولخص أن البكتريا المعوية في الماشية والدجاج التي تعيش على مقربة من البشر تتطلب إجراءات مراقبة أفضل للحماية الحيوانات والبشر المعرضين للخطر.

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